

Stromal Cell-Derived Factor-1 from Biliary Epithelial Cells Recruits CXCR4-Positive Cells: Implications for Inflammatory Liver Diseases

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SUMMARY: Although stromal cell-derived factor-1 (SDF-1) plays an important role in hematopoiesis in the fetal liver, the role after birth remains to be clarified. We investigated the role of SDF-1 and its receptor, CXCR4, in 75 patients; this included controls and patients with viral hepatitis, liver cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis. Interestingly, SDF-1 appeared up-regulated in biliary epithelial cells (BEC) of inflammatory liver disease. Furthermore, in inflammatory liver diseases, SDF-1 was expressed by BEC of interlobular and septal bile ducts and by proliferated bile ductules. The message expression of SDF-1 in BEC was confirmed at a single-cell level by RT-PCR and laser capture microdissection. The plasma levels of SDF-1 were significantly higher in patients with liver diseases than in normal controls. Flow cytometric analysis of the surface expression of CXCR4 showed that most liver-infiltrating lymphocytes express CXCR4 and the intensity was up-regulated more significantly in liver-infiltrating lymphocytes than in peripheral blood lymphocytes. These results suggest that increased SDF-1 production by BEC may play an important role in the recruitment of CXCR4-positive inflammatory cells into the diseased livers. These data are significant because modulation of the SDF-1/CXCR4 interaction has therapeutic implications for inflammatory liver diseases. (*Lab Invest* 2003, 83:665-672).

Immune competent cells such as dendritic cells and lymphocytes continuously migrate from blood to lymphoid or nonlymphoid tissues in both physiologic and pathologic conditions (von Andrian and Mackay, 2000; Westermann et al, 2001). In the process of migration and homing, immune cells must adhere and penetrate blood vessels. Adhesion molecules such as selectins, integrins, and Ig superfamily play an important role in tethering and rolling of circulating cells. Chemokines are chemoattractants that bind to specific surface receptors of immune cells and transmit signals. More than 50 chemokines and 18 chemokine receptors have been identified, and the type and distribution of chemokines and the type of receptors are orchestrated to pursue appropriate migration and recruitment of immune cells (Cyster, 1999; Kim and Broxmeyer, 1999; Melchers et al, 1999).

In viral hepatitis and autoimmune liver diseases, many inflammatory cells accumulate in the portal tract, which is regarded as the secondary lymphoid

tissue (Grant et al, 2002; Yoneyama et al, 2001). These inflammatory cells include not only antigen-specific T cells but also nonspecifically activated immune cells. Chemokines and chemokine receptors are known to play an important role in the accumulation of inflammatory cells in liver diseases (Grant et al, 2002; Kusano et al, 2000; Marra et al, 1998; Narumi et al, 1997; Nishioji et al, 2001; Shields et al, 1999; Shimizu et al, 2001; Tamaru et al, 2000; Tsuneyama et al, 2001; Yoneyama et al, 2001). Chemokines such as IFN-inducible protein-10, monokine-induced IFN- γ , and macrophage inflammatory protein-1 (MIP-1) have been suggested to play a role in the accumulation of T cells in the human liver of viral hepatitis and autoimmune hepatitis (AIH).

Within the portal tract, inflammatory cells accumulate either at the interface between the portal tract and the parenchyma or around the bile duct. The accumulation of inflammatory cells around the bile duct is a characteristic feature of primary biliary cirrhosis (PBC), but the same can be seen in livers of viral hepatitis or AIH (Czaja and Carpenter, 2001; Kaji et al, 1994; Ludwig et al, 1984). The accumulation of inflammatory cells around the bile ducts in PBC has been explained as immunologic reactions against biliary epithelial cells (BEC). Increased expression of adhesion molecules such as intercellular adhesion molecule-1 has been demonstrated in BEC of PBC and may partici-

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pate in the recruitment of leukocyte function-associated antigen-1–positive inflammatory cells (Mosnier et al, 1994; Yasoshima et al, 1995).

However, it is not known what mechanisms are involved in the accumulation of inflammatory cells in the portal tracts or around the bile ducts in viral hepatitis or AIH. In a previous study, B lineage cells in addition to T cells have been demonstrated to accumulate around the bile ducts of various liver diseases including PBC (Nakanuma, 1993). It is suggested that BEC might mobilize inflammatory cells through chemoattraction by producing some chemokines. Stromal cell–derived factor-1 (SDF-1) is a chemokine that is essential for the maturation of premature B cells (Coulomb-L'Hermin et al, 1999). Moreover, in the fetal liver, SDF-1 is produced by ductal plate cells, which are progenitor cells of BEC (Coulomb-L'Hermin et al, 1999). SDF-1 is not only a chemokine that is important for the development and maturation of B lineage cells but is also a chemoattractant for T cells, premature B cells, and monocytes (Bleul et al, 1996; Ma et al, 1998; Nagasawa et al, 1996; Nanki et al, 2000; Poznansky et al, 2000; Voermans et al, 2001).

The sole receptor for SDF-1 is CXCR4, which is expressed on T and B cells and on monocytes (Ma et al, 1999; Mohle et al, 1998; Nagasawa, 2000). CXCR4 is also essential for hematopoiesis and B-cell maturation, and the CXCR4 knockout mice results in fetal

death with disturbed hematopoiesis as in SDF-1 knockout mice. In vitro, CXCR4-positive cells show chemoattraction to SDF-1. We report a selective increased SDF-1 production by BEC that leads to recruitment of CXCR4-positive cells in inflammatory liver pathology. These results have several implications, including the potential for disease modulation.

Results

SDF-1 Protein Expression in BEC

Immunohistochemical study showed negative or faint expression of SDF-1 protein in BEC of normal livers (Fig. 1a). On the other hand, most BEC of diseased livers showed intense expression of SDF-1 (Fig. 1, b to d). The expression of SDF-1 was restricted to BEC, and hepatocytes were negative. Expression was not seen in any other cell component in the portal tract or in the parenchyma.

Reactive bile ductules in viral hepatitis or autoimmune liver diseases also showed the expression in various degrees. Reactive bile ductules were observed mainly at the interface between the portal tract and liver parenchyma and were numerous in patients with interface hepatitis or with Stages 3, 4, and 5. The grade of ductular proliferation and intensity of SDF-1 expression varied among portal tracts even in the

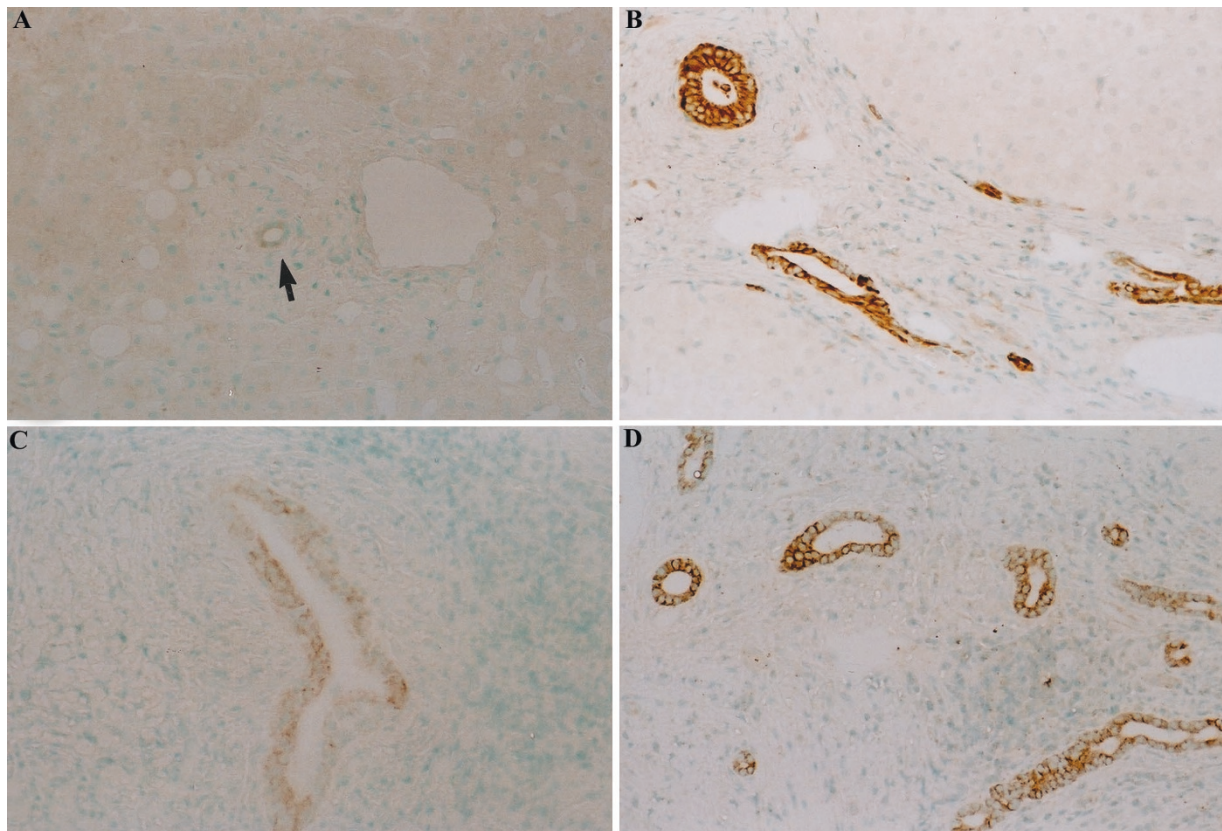


Figure 1.

Immunohistochemical staining of stromal cell–derived factor-1 (SDF-1). a, Normal liver bile duct. SDF-1 is faintly expressed in some biliary epithelial cells (BEC) (arrow). Intense expression of SDF-1 is observed in BEC of autoimmune hepatitis (AIH) (b), primary biliary cirrhosis (PBC) (c), and chronic hepatitis C (CHC) (d). Proliferated bile ductules are also positive (d). Magnification, $\times 160$.

same patient. The staining was more intense in interlobular or septal bile ducts than in reactive bile ductules. The intensity of SDF-1 expression tended to be stronger in patients with advanced fibrosis than in those with less advanced fibrosis. However, the pattern of SDF-1 expression did not differ between viral and autoimmune liver diseases.

SDF-1 Message Expression

We then examined the message expression of SDF-1 in normal and diseased livers. RT-PCR using specific primers for SDF-1 demonstrated positive messages in liver samples from normal and diseased livers (Fig. 2a). To further study whether BEC express the SDF-1 message, we selectively collected BEC by laser capture microdissection and amplified the message with RT-PCR. BEC in diseased livers also showed a positive SDF-1 message (Fig. 2b). Hepatocytes were negative for the SDF-1 message in both normal and diseased livers.

SDF-1 Levels in Plasma

Plasma levels of SDF-1 are shown in Figure 3. The plasma levels of SDF-1 in normal controls were 1792 ± 365 pg/ml (mean \pm SD). The plasma levels of patients with autoimmune liver disease and viral liver disease were 2260 ± 550 pg/ml and 2374 ± 418 pg/ml, respectively. The plasma level was significantly higher in patients with autoimmune and viral liver diseases than in normal controls ($p < 0.05$ and $p < 0.01$, respectively). There was no significant difference in the plasma SDF-1 levels among the types of liver disease. The plasma levels of SDF-1 did not show any correlation with serum levels of alanine aminotransferase, alkaline phosphatase, or bilirubin.

CXCR4 Expression in Liver-Infiltrating Lymphocytes (LIL)

To study whether inflammatory cells in the liver show the receptor for SDF-1, we then examined the surface expression of CXCR4 in LIL. The frequency and inten-

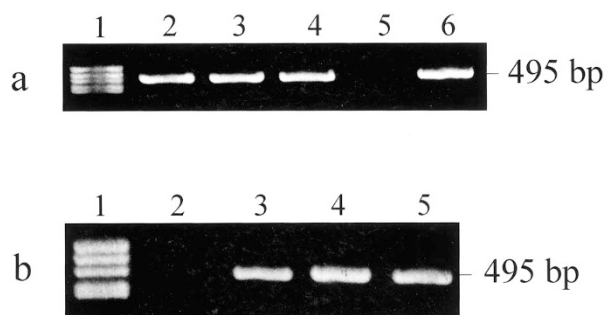


Figure 2.

Message expression of SDF-1 by RT-PCR. a, RT-PCR of SDF-1 in normal and diseased liver tissues. The SDF-1 message is expressed in normal and diseased livers. Lane 1, DNA size marker; lane 2, normal liver; lane 3, liver cirrhosis B (LCB); lane 4, PBC; lane 5, negative control; lane 6, positive control. b, RT-PCR of laser-captured BEC. SDF-1 is selectively expressed in BEC. Lane 1, DNA size marker; lane 2, normal liver hepatocytes; lane 3, normal liver BEC; lane 4, BEC from PBC liver; lane 5, BEC from LCB.

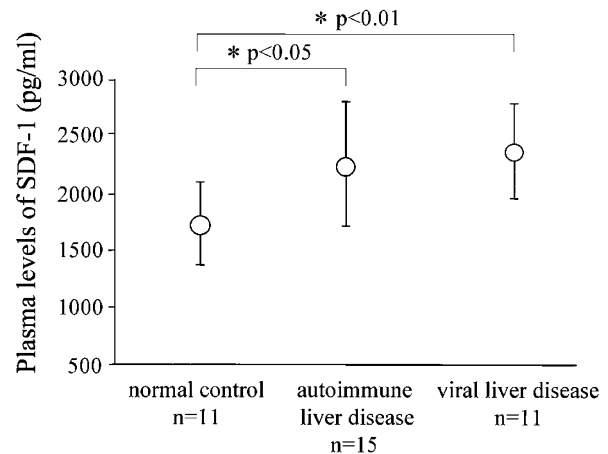


Figure 3.

Plasma levels of SDF-1. Plasma SDF-1 levels are significantly higher in autoimmune or viral liver diseases than in normal controls (mean \pm SD, $p < 0.05$ and $p < 0.01$, respectively).

sity of CXCR4 expression were studied by flow cytometry and compared between LIL and peripheral blood lymphocytes (PBL) (Fig. 4). More than 90% of lymphocytes were positive for CXCR4 in both LIL and PBL, and the frequency of CXCR4-positive cells was not different. However, the CXCR4 intensity of LIL was higher than that of PBL. The intensity was analyzed in T cell (CD3⁺) and B cell (CD19⁺) populations. In both populations, LIL showed higher intensity than PBL (Fig. 4b).

Phenotype of Inflammatory Cells Around SDF-1-Positive Bile Ducts

The phenotypes of inflammatory cells infiltrating around SDF-1-positive bile ducts were studied in serial sections. CD4 T cells, CD8 T cells, and CD79 α -positive B lineage cells were accumulated around SDF-1-positive bile ducts (Fig. 5, a to d).

Discussion

The present study demonstrated that BEC of the human liver produce a chemokine, SDF-1, the expression of which was augmented in diseased livers compared with that in normal livers. Serum levels of SDF-1 were also elevated in patients with liver diseases. Moreover, most T and B cells accumulated in the liver showed surface expression of the chemokine receptor, CXCR4, and the expression was markedly up-regulated on LIL compared with that on PBL. These results suggest that the SDF-1/CXCR4 interaction may play a role in the accumulation of inflammatory cells in the portal tract of viral and autoimmune liver diseases.

Previously, SDF-1/CXCR4 interactions were suggested to be involved in normal homeostasis such as hematopoiesis, vascular development, or homing of naive T cells to secondary lymphoid organs (Bleul et al, 1996; Coulomb-L'Hermin et al, 1999; Ma et al, 1998, 1999; Mohle et al, 1998; Nagasawa, 2000;

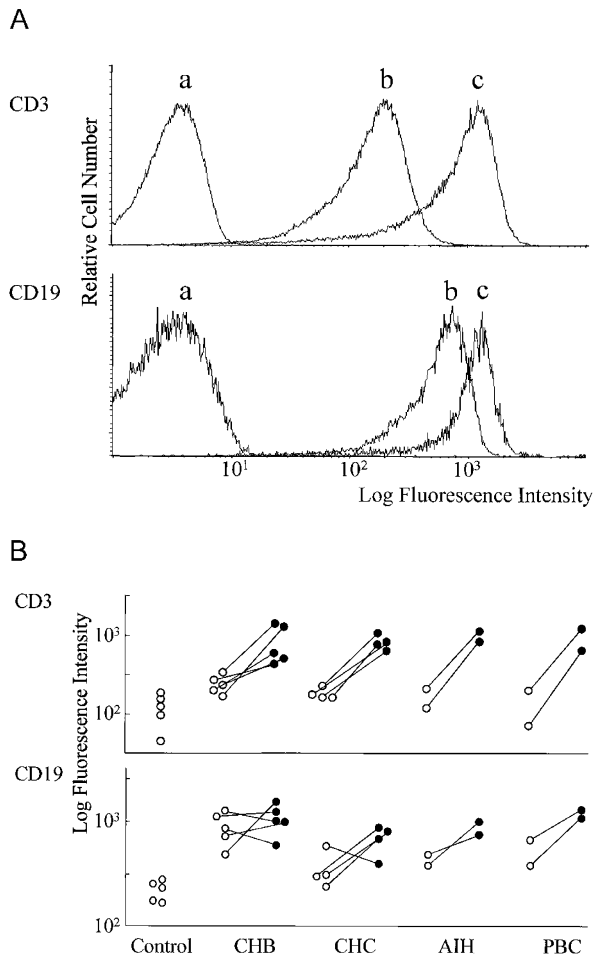


Figure 4.

CXCR4 expression in liver-infiltrating lymphocytes (LIL) and peripheral blood lymphocytes (PBL). a, The intensity of CXCR4 was examined separately in CD3⁺ (upper panel) and CD19⁺ (lower panel) fractions. CXCR4 is up-regulated in both fractions of LIL compared with those in PBL. a, Isotype control; b, PBL; c, LIL. b, Comparison of CXCR4 intensity between PBL (open circles) and LIL (closed circles) in normal controls and patients with chronic hepatitis B (CHB), CHC, AIH, and PBC. CD3⁺ cells in LIL showed increased intensity of CXCR4 compared with those in PBL. CD19⁺ cells in LIL also showed increased intensity compared with those in PBL. In CHB, CXCR4 intensity was high in PBL and in LIL.

Nagasawa et al, 1996; Nanki et al, 2000; Poznansky et al, 2000; Sallusto et al, 1998; Voermans et al, 2001). It has been assumed that SDF-1 gene expression is constitutive in a number of tissues, because the promoter of *sdf1* contains several CpG islands, a transcription factor binding motif commonly observed in housekeeping genes, and binding motifs for the transcription factors nuclear factor kappa B (NF- κ B) and activator protein-1 (AP-1) have not been found in a 19-kb *sdf1* genomic clone (Shirozu et al, 1995). The present findings suggest an additional new role for SDF-1/CXCR4 interactions.

Roles for the SDF-1/CXCR4 interaction, other than normal homeostasis and development, have been reported recently in pathologic conditions such as rheumatoid arthritis (RA) (Nanki and Lipsky, 2000), inflammatory skin diseases (Pablos et al, 1999), and skin wound healing (Fedyk et al, 2001). In the RA

synovium, SDF-1 was expressed in cells with a fibroblastic appearance, whereas it was not expressed in the synovium of osteoarthritis. CD40 engagement using anti-CD40 mAb enhanced the production of SDF-1 by synovial fibroblasts, suggesting that CD40 ligand expressed by CD4 T cells in the RA synovium may stimulate SDF-1 production and recruit CXCR4-positive T cells into the inflamed tissues.

Controversial results have been reported on the SDF-1 production of skin fibroblasts. Pablos et al (1999) reported that SDF-1 was expressed in fibroblasts close to or within dermal inflammatory infiltrates of inflammatory skin diseases, whereas it is confined to endothelial cells, pericytes, and dendritic cells, not fibroblasts, in normal human skin, suggesting that SDF-1 gene expression of skin fibroblasts is up-regulated by proinflammatory stimuli. In contrast, Fedyk et al (2001) demonstrated that SDF-1 production by skin fibroblasts is inhibited by cytokines such as IL-1 and TNF- α . Down-regulation of the SDF-1 production is explained as a mechanism to inhibit monocyte infiltration in the process of wound healing. They further suggested that ligation of CD40 on fibroblasts by CD40 ligand may trigger down-regulation of SDF-1 production. Although the mechanism of up- or down-regulation of SDF-1 production remains unknown, these findings suggest that SDF-1 expression is not merely constitutive but is also controlled by other circumstantial stimuli.

The present study demonstrated enhanced SDF-1 expression on BEC in diseased livers compared with that in normal liver, although the previous report showed SDF-1 expression on BEC in the normal liver as well as livers undergoing rejection (Goddard et al, 2001). A low level of expression may exist on BEC in the normal liver, as the message is demonstrated in the normal liver. The mechanism by which BEC up-regulate the SDF-1 expression remains to be clarified. Proinflammatory stimuli from inflammatory cells may play a role in the induction of SDF-1 production by BEC. As shown in RA synovial fibroblasts and skin fibroblasts, BEC have been demonstrated to express CD40 (Cruickshank et al, 1998). Ligation of CD40 on BEC is shown to induce apoptosis through the activation of NF- κ B and AP-1 (Afford et al, 2001). Although binding motifs for NF- κ B and AP-1 have not been found in SDF-1, CD40 ligands expressed on infiltrating T cells may trigger the expression by modifying intracellular signaling. Cholestasis, including bile acid retention, might be another mechanism for the expression of SDF-1 because biliary constituents are reabsorbed from BEC and stimulate the gene expression of BEC (Hirano et al, 2001).

The other possible mechanism of SDF-1 production in BEC might be phenotypic dedifferentiation and reactivation of the gene in the progenitor cells, because ductal plate cells, progenitor cells of BEC, produce SDF-1 in the developing liver. It is conceivable that the phenotype of matured BEC may change to that of immature or dedifferentiated cells under pathologic conditions and BEC may regain the ability to produce SDF-1.

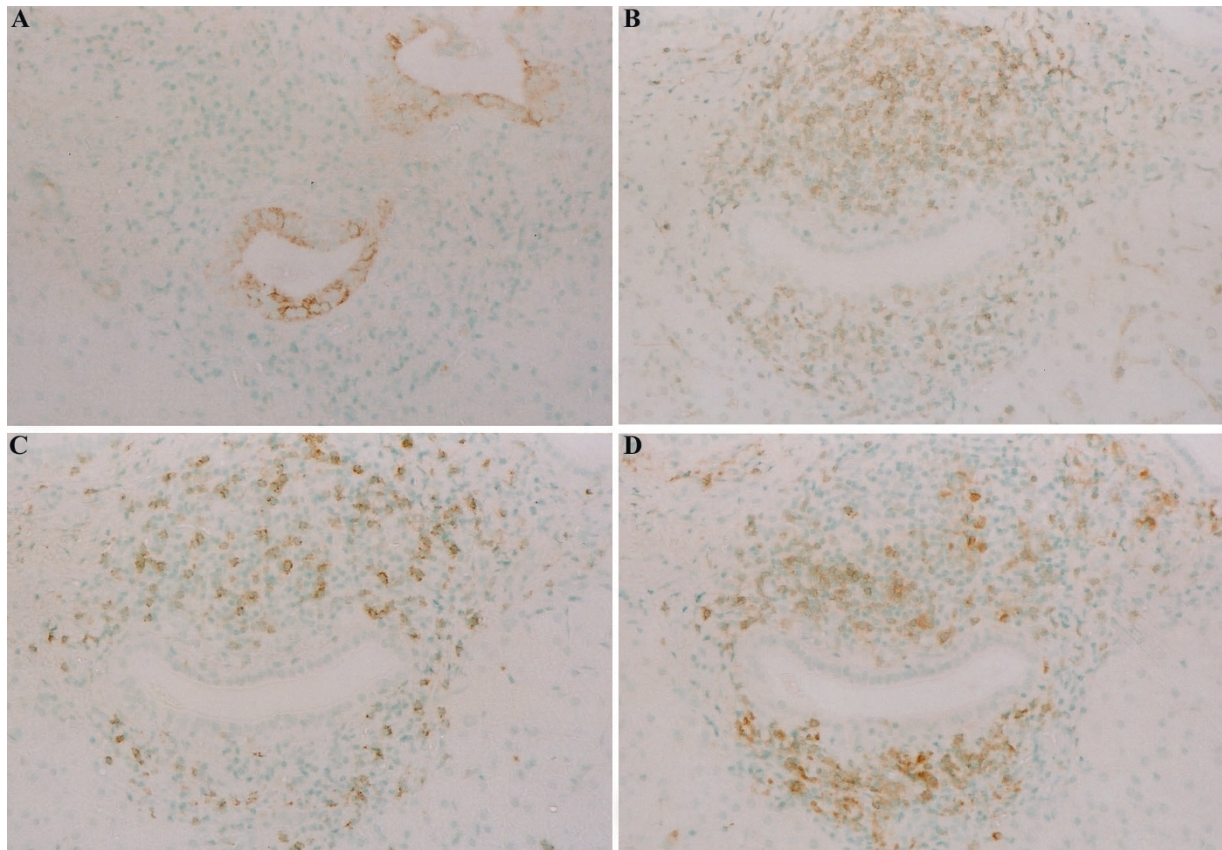


Figure 5.

Immunohistochemical staining of SDF-1 (a), CD4 (b), CD8 (c), and CD79 α (d) in serial sections of PBC liver. CD4 $^{+}$, CD8 $^{+}$, and CD79 α^{+} cells were accumulated around SDF-1-positive bile ducts. Magnification, $\times 160$.

CXCR4 is a sole receptor for SDF-1 and is expressed on monocytes, B cells, and naive T cells among peripheral blood cells. The expression of CXCR4 on LIL is enhanced compared with that on PBL. Intense expression of CXCR4 on LIL can be explained either by selective chemoattraction of CXCR4-positive cells through SDF-1 or by up-regulation in the liver. CXCR4 is rapidly up-regulated after both PHA stimulation and IL-2 priming. In the RA synovium, T cells are induced to express CXCR4, and transforming growth factor β is demonstrated to be responsible for the expression (Buckley et al, 2000). These CXCR4-positive synovial T cells show better adherence to fibronectin in response to SDF-1 than PBL. Similar mechanisms may work in liver diseases, although this needs to be clarified.

Recently, the important role of chemokine and chemokine receptor interactions in the migration of inflammatory cells into the liver were reported. Secondary lymphoid chemokine (CCL21) up-regulated on the vascular endothelium in portal-associated lymphoid tissue plays an important role in the migration of CCR7-positive mucosal lymphocytes into the portal tract of primary sclerosing cholangitis (Grant et al, 2002). In chronic hepatitis C (CHC)-infected liver, MIP-1 α and MIP-1 β expressed in portal tract vessels recruit CCR5-positive lymphocytes into the portal tract, whereas IFN-inducible protein-10 and

monokine-induced IFN- γ expressed in the sinusoidal endothelium recruit CXCR3-positive lymphocytes into parenchyma (Shields et al, 1999). SDF-1/CXCR4 interactions shown in the present study may play a role in the migration and recruitment of CXCR4-positive lymphocytes around the bile ducts. These results suggest that each pair of combination recruits a distinct lymphocyte subpopulation into a specific compartment in the liver. This is supported by the recent findings that differential expression of chemokines and chemokine receptors shape inflammatory response in the rejecting human liver transplant (Goddard et al, 2001).

Up-regulation of SDF-1 was not restricted to any specific liver disease. The lack of disease specificity may raise some concern about the functional significance of SDF-1. However, the lack of disease specificity has been shown in other chemokine/chemokine receptor interactions, in which signals such as NF- κ B and AP-1 induced by inflammation up-regulate the expression. Although the mechanism of SDF-1 up-regulation is not known, the trigger for up-regulation might be a ubiquitous process rather than a disease-specific one.

Two types of BEC exist in diseased livers and both produce SDF-1: one is BEC of interlobular or septal bile ducts and the other is BEC of reactive bile ductules near the canal of Hering, which may contain bipotential progenitor cells of the liver. The pattern of

inflammatory cell accumulation in the portal tract is distinct among types of liver diseases. In PBC, inflammatory cells accumulate around the interlobular or septal bile duct, whereas they accumulate at the interface between portal tracts and liver parenchyma in AIH. The location of the SDF-1/CXCR4 interaction may determine the pattern of inflammatory cell accumulation. SDF-1 produced by reactive bile ductules may induce migration at the interface, whereas that produced by the interlobular bile duct may induce periductal accumulation of inflammatory cells. Although the present study failed to delineate the role of two types of BEC due to varying degrees of reactive bile ductules and heterogeneous expression of SDF-1, functional studies are needed to clarify the role of BEC in inflammatory cell accumulation in the liver.

In summary, the data presented demonstrate increased SDF-1 production in inflammatory liver disease as well as the expression of CXCR4 in LIL, suggesting an important role of the SDF-1/CXCR4 interaction in lymphocyte accumulation in the liver. Although the SDF-1/CXCR4 interaction is not the sole chemokine involved in the liver inflammation, the universal expression of SDF-1 in various inflammatory liver diseases provides a mechanism for future modulation of the SDF-1/CXCR4 interaction.

Materials and Methods

Subjects

All patients were admitted to our hospital, and liver biopsy was performed for the diagnosis of liver diseases. Written informed consent was obtained from the subjects, and the study was approved by the Institutional Committee for Human Rights. No patients received prior specific treatments. Liver biopsy tissues were obtained from 75 patients: 11 patients with chronic hepatitis B (CHB), 28 with CHC, 4 with liver cirrhosis B (LCB), 4 with liver cirrhosis C (LCC), 19 with PBC, 2 with primary sclerosing cholangitis, and 7 with AIH.

Histologic findings of viral hepatitis and AIH were evaluated using a modified histologic activity index (Ishak et al, 1995). Histologic staging of viral hepatitis showed nine patients with Stage 1, nine with Stage 2, nine with Stage 3, eight with Stage 4, and four with Stage 5; staging of AIH showed two patients with Stage 1, two with Stage 2, two with Stage 3, and one with Stage 4. Histologic findings of PBC patients included 13 patients with Stage 1, 3 with Stage 2, and 1 with Stage 3 with Scheuer's classification (Scheuer, 1967).

Ten normal liver tissues were obtained from donors for liver transplantation and from patients with cholelithiasis or stomach cancer for the diagnosis of liver disease.

Liver Tissues

A part of the liver tissues was fixed in 10% buffered formalin and embedded in paraffin for routine histology and immunohistochemical examination. A part

was stored in a solution containing 4 M guanidine thiocyanate and 0.1 M 2-mercaptoethanol for RNA extraction and message analysis. Other parts were frozen in Tissue-Tek OCT compound (Miles, Inc., Elkhart, Indiana) for laser capture microdissection.

Immunohistochemistry

Five-micrometer sections were prepared from paraffin-embedded samples and used for immunohistochemical staining of SDF-1, as described previously (Yabushita et al, 2001). Briefly, sections were pretreated at 100° C in a microwave oven in 0.1 M sodium citrate solution for 10 minutes and incubated with mAb for SDF-1 (R&D Systems, Minneapolis, Minnesota) overnight at 4° C after nonspecific binding was blocked with 10% goat serum (Nichirei, Tokyo, Japan). Intrinsic peroxidase activity was blocked in a methanol solution containing 0.3% hydrogen peroxide, and the sections were treated with EnVision (Dako Company, Carpinteria, California). Finally, the sections were immersed in 3,3'-diaminobenzidine, and nuclear staining was performed with 5% methyl green. For the control experiment, the first mAb was omitted from the reaction procedure.

Serial sections were prepared and stained for CD4 T cells (anti-CD4, MT310; Dako Company), CD8 T cells (anti-CD8, clone DK25; Dako Company), and B lineage cells (anti-CD79 α , clone JCB117; Dako Company) with the method described above.

Laser Capture Microdissection

Ten-micrometer frozen sections were prepared, fixed in a 70% ethanol solution, and stained with hematoxylin and eosin. The sections were dehydrated through a graded series of ethanol and xylene and air-dried. BEC, approximately 100 cells, were selectively picked up by Laser Capture Microscopy (Arcturus Engineering, Inc.) and collected into tubes for RNA extraction.

RT-PCR

Total RNA was extracted with the Strataprep Total RNA Microprep kit (Stratagene, La Jolla, California), and cDNA was prepared using the SuperScript First-Strand Synthesis System for RT-PCR (Life Technologies, Gaithersburg, Maryland). A 1- μ l aliquot of the cDNA reaction product was mixed with primers specific for SDF-1 (sense 5'-GGGCATGGATGAATATAAGCTGC-3', antisense 5'-CCATGAACGCCAAGGTCGTGGTC-3'). After preheating at 95° C for 10 minutes, PCR was performed with 200 μ mol of dNTP and 1.25 U of AmpliTaq Gold (Perkin Elmer, Foster City, California) for 45 cycles (94° C for 1 minute, 60° C for 1 minute, 72° C for 1 minute) and a final extension (72° C for 10 minutes) in a Perkin-Elmer/Cetus thermocycler (Perkin Elmer Japan Company, Chiba, Japan). The PCR product (4 μ l) was loaded on a 2% agarose gel with ethidium bromide and visualized by UV fluorescence.

Plasma Levels of SDF-1

Plasma levels of SDF-1 were measured with an ELISA assay kit (Quantikine; R&D Systems). Plasma was obtained from 11 normal subjects and patients with viral hepatitis (CHB, $n = 2$; CHC, $n = 8$; and LCC, $n = 1$), PBC ($n = 8$), and AIH ($n = 6$).

Preparation of Liver-Infiltrating and PBL

LIL and PBL were obtained from patients with CHB ($n = 5$), CHC ($n = 4$), PBC ($n = 2$), and AIH ($n = 2$). PBL were also obtained from five normal subjects. LIL was isolated from biopsy samples after collagenase digestion and Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Heparinized peripheral blood was obtained at the time of liver biopsy, and PBL were isolated by Ficoll-Hypaque (Pharmacia) gradient centrifugation. PBL and LIL were analyzed immediately by flow cytometry.

Flow Cytometry

We analyzed the surface expression of CXCR4 in both LIL and PBL by flow cytometry. The mAbs used for this study were directly coupled to FITC or PerCP. The following mAbs were used: anti-CD3 (SK-7)-FITC, anti-CD19-FITC (as a B-cell marker), and anti-CXCR4-FITC (Becton Dickinson, San Jose, California). Approximately 5×10^5 lymphocytes from LIL or PBL were stained with mAbs, as described above, and analyzed in a three-color FACScan flow cytometer (Becton Dickinson). All flow cytometry findings were analyzed with CELLQuest software (Becton Dickinson). The expression and intensity of CXCR4 were analyzed separately in T- or B-cell populations and compared between LIL and PBL.

Statistical Analysis

Plasma levels of SDF-1 were compared among groups of normal subjects, viral liver diseases, and autoimmune liver diseases using Student's *t* test.

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